The daily periodicity of cell-division and of elongation in the root of Allium

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1. Introduction

It is a well-known fact that rhythmic variation in the rate of growth is widespread, perhaps universal. It is similarly known that the nature of the rhythm varies considerably in different plants, so that there is no uniform hour at which the rate of growth is highest or lowest: each form seems to have its own peculiar rhythm. Perhaps this is one reason why there is at present so little uniformity of opinion among those who have studied the phenomena of growth in various plants, as to the factors involved in the production and maintenance of this rhythm. One possible factor has been overlooked almost entirely, namely, that of cell-division. As a matter of fact very little is known concerning the actual relations between cell-division and growth. Practically the only record of any observed correlation between cell-division and elongation is that of Ward,* who observed that, in *Bacillus ramosus*, "the period of cell-division entails more or less cessation of growth" (page 294), and that (pages 301–2) "periods

*Ward, H. M. On the biology of *Bacillus ramosus* (Fraenkel), a schizomycete of the river Thames. Proc. Roy. Soc. **58** : 265–468. 1895.

of slowest growth . . . were the approximate moments of insertion of the septa, and consequently, moments when elongation would be going on with the least rapidity. . . ." Concerning the correlation of these processes in higher forms nothing at all is known.

Not only has there been no real attempt to correlate the processes of elongation and cell-division in a given organ, but there is a complete lack of knowledge concerning the course of cell-division alone in a growing part. And while much is known with regard to the daily rhythm of elongation or growth of organs, in nearly every instance the organ observed has been aërial and therefore subject to very profound and rapid environmental disturbances accompanying the alternating conditions of day and night. As far as can be discovered the only observations on the daily periodicity of growth of subterranean parts are those of Strehl* on the roots of *Lupinus*, and MacMillan † on the potato-tuber.

It seems, therefore, that here is an opportunity for profitable investigation: to determine whether there is a rhythmic daily variation in the rate of cell-division of a growing organ; to determine whether there is a definite daily rhythm in the growth or elongation of a subterranean part which is growing under much more uniform conditions than the aërial parts; and finally, if such rhythms are found to exist, to determine whether there is any correlation between the processes of cell-division and elongation. The observations described in this paper were designed to attack these questions, and it is believed that the results have been definite enough at least to warrant further serious investigation.

I wish to take this opportunity to express my very considerable indebtedness to Dr. C. C. Curtis, Columbia University, under whose direction these observations were carried out, for his ever ready aid and valuable counsel.

2. Summary

The principal facts brought out in this paper are the following:

1. In the root of *Allium* there are two maxima and two minima in the rate of cell-division during twenty-four hours.

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* Strehl, R. Untersuchungen über das Längenwachsthum der Wurzel und des hypokotylen Gliedes. Leipzig, 1874.

† MacMillan, C. On the growth-periodicity of the potato-tuber. Am. Nat. **25**: 462–469. 1891.

2. The primary maximum occurs shortly before midnight (11 p. m.) and the primary minimum about 7 a. m. The secondary maximum occurs about 1 p. m. and the secondary minimum about 3 p. m.

3. There is no correspondence between the rate of cell-division and slight variations in temperature.

4. In the root of *Podophyllum*, maximal and minimal points occur at almost the same hours as in *Allium*.

5. Tap-water alone or with various substances in solution, seriously affects the course of cell-division. In some cases there may be recovery and partial return to the normal condition.

6. Under normal conditions of growth, the rate of elongation of the root of *Allium* exhibits a daily rhythm showing two maxima and two minima during twenty-four hours.

7. Elongation is most rapid (primary maximum) about 4 or 5 p. m., the secondary maximum occurring about 7 a. m. The primary minimum is about 11 p. m. and the secondary minimum about noon.

8. Periods of rapid cell-division coincide with low rate of elongation, and during rapid elongation the rate of cell-division is lowest.

3. Methods

(*a*) CELL-DIVISION

Sound average-sized bulbs of *Allium* were selected and allowed to germinate at ordinary room-temperature in moist sand or pine sawdust until the roots were from 50 to 100 mm. in length: this was usually after from four to six days. Then at definite intervals, usually of about two hours, throughout 24-hour periods, two root-tips from different parts of the bulb were taken for examination. The tips were fixed 20 minutes in acetic-alcohol (glacial acetic 1 part, 70 per cent. alcohol 2 parts), sectioned longitudinally 10 microns thick, and stained in Delafield's haematoxylin.

It is believed that the method used in determining the relative numbers of dividing cells in the different tips entirely obviated error due to differences in the size of the tips. From each, the three sections passing nearest the axis of the root were selected and the area to be examined determined in the following way. At

a distance of 5 micrometer spaces (eye-piece micrometer ruled in millimeters, ocular 3, objective Zeiss A, tube-length 140 mm.) from the growing point of the tip (the root has attained a uniform diameter at this point) its diameter was measured carefully. An imaginary line was then drawn across the section at a distance from its extremity of twice its diameter. The area examined was that below this transverse line (FIGURE 1), and was found in every instance to contain practically all of the actively dividing cells of the undifferentiated tissue.

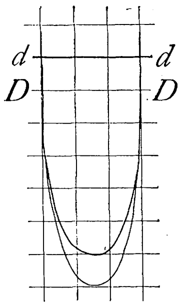

FIGURE 1. Diagram showing method of measurement. *DD*, diameter 5 spaces from apex. Area examined extended to line *dd*. Area of this tip = 15.

The number of dividing cells within the corresponding areas of the three selected sections was then carefully counted. The dividing cells were tabulated in three groups. The first group included those cells in the early stages of mitosis up to the time of the formation of the equatorial plate, *i. e.*, all stages of the prophase. The second group included those cells in metaphase and early anaphase up to the time when the divergence of the chromosomes is complete. The last group included the cells in late anaphase up to the time of the reconstruction of the nucleus and formation of the new cell-wall. These groups were then added and the average of the three sections taken as the number characteristic of the tip.

In order to avoid error due to the comparison of tips of different sizes the areas of the sections were reduced to a common area and the proportional numbers of dividing cells calculated. The average area of the sections was about fifteen micrometer squares; therefore the numbers of cells in sections having larger or smaller areas than this were calculated proportionally.

This method of comparison is justified by the fact that the size of a part or organ is determined by the number of cellular elements contained in it and not by variations in their size.* Several counts were made in given areas of large and small root-tips of *Allium*, and this was found to be true.

* Amelung, E. Ueber mittlere Zellengrösse. Flora, 77: 176. 1893.—Strasburger, E. Histologische Beiträge. 5: 117–118. 1893.

The series of *Podophyllum* used as a check were treated in the same manner except that the smaller size of these tips necessitated their reduction to an average area of nine micrometer spaces.

(*b*) ELONGATION

In determining the periodicity of elongation the method of direct observation was used. Onions were grown in pine sawdust in pots with a narrow glass slip set in one side. Roots frequently grew almost vertically along this glass and would be exposed properly to moisture and air in the pot. These bulbs were of course grown entirely in the dark-room. When the roots were about 35–40 mm. long they were observed by candle-light through a horizontal microscope and the temperature recorded, at one- or two-hour intervals during a 24-hour period.

4. Daily periodicity of cell-division

A. UNDER NORMAL CONDITIONS

(*a*) *Typical observation*.—We shall consider first the rhythm of cell-division under normal circumstances of growth. To illustrate more fully the method employed and to furnish a basis for comparison, one of the records has been reproduced completely in TABLE 1.

This table shows that by far the larger proportion of cells in the process of mitosis were in the prophase, as would be expected, since the preparation for and inauguration of mitosis occupy a much longer period than the later phases, which are passed through very rapidly. The table shows also how remarkably similar are the conditions of the root-tips examined at the same hour. This similarity is much closer than was expected on account of the numerous factors liable to cause individual variations. It will be seen that at 9 a. m., 11 a. m. and 1 p. m., for example, the numbers of dividing cells are almost identical in the two tips, while only in a few instances, such as at 5 p. m., 9 p. m. or 1 a. m., were there differences at all considerable.

The final averages of this table are represented in the form of a curve in FIGURE 2, *curve I*, which shows graphically the rate of cell-division during a 24-hour period. The curve is remarkably regular, with two maxima and two minima. Cell-division is most rapid (primary maximum) at 11 p. m., the secondary maximum

TABLE I

NUMBERS OF DIVIDING CELLS IN ROOTS OF SINGLE BULB OF ALLIUM

Time.	Temperature.	Area examined.	Dividing cells.			Total dividing cells.	Total reduced to area=15.	Average for each tip.	Average of two tips.
			Early.	Middle.	Late.				
9 a. m.	16°	11.5	25	9	1	35	46	47	47
			23	8	4	35	46		
			30	5	3	38	50		
		13.5	33	3	3	39	43	47	
			36	2	4	42	47		
			37	3	5	45	50		
11 a. m.	17°	12.5	28	10	6	44	53	54	54
			33	8	6	47	56		
			27	9	8	44	53		
		12—	28	7	2	37	47	53	
			38	11	4	53	60		
			24	5	4	33	45		
1 p. m.	17.5°	13	46	4	4	54	62	61	62
			47	2	2	51	59		
			49	2	2	53	61		
		15.5	58	4	6	68	66	63	
			53	3	8	64	62		
			49	4	9	62	60		
3 p. m.	27°	12.5	26	2	3	31	37	39	35
			29	1	2	32	38		
			33	1	1	35	42		
		12	23	0	3	26	33	31	
			21	0	3	24	30		
			21	1	1	23	29		
5 p. m.	19°	13.5+	24	3	3	30	33	33	50
			26	2	2	30	33		
			25	2	2	29	32		
		11.5	37	4	6	47	61	67	
			46	1	3	50	65		
			45	6	6	57	74		
7 p. m.	17°	12	43	2	9	54	67	65	62
			39	4	8	51	64		
			38	3	10	51	64		
		13	42	2	4	48	55	59	
			47	4	4	55	63		
			45	1	6	52	60		
9 p. m.	15°	14	47	6	1	54	58	67	75
			53	8	4	65	70		
			65	2	0	67	72		
		13+	64	2	3	69	79	83	
			72	2	3	77	88		
			65	3	4	72	82		
11 p. m.	14.5°	11.5	65	3	4	72	94	93	89
			64	5	7	76	99		
			60	3	4	67	87		
		13	62	3	3	68	78	86	
			70	4	5	79	91		
			69	3	4	76	88		

TABLE I—*Continued*

NUMBERS OF DIVIDING CELLS IN ROOTS OF SINGLE BULB OF ALLIUM

Time.	Temperature.	Area examined.	Dividing cells.			Total dividing cells.	Total reduced to area=15.	Average for each tip.	Average of two tips.
			Early.	Middle.	Late.				
1 a. m.	14°	13—	37	1	1	39	45	48	57
			38	2	3	43	50		
			33	4	5	42	48		
		11+	45	5	2	52	69	66	
			40	2	3	45	60		
			44	5	2	51	68		
3 a. m.	14°	15	31	2	2	35	35	40	42
			35	3	2	40	40		
			35	8	1	44	44		
		17+	48	1	6	55	47	44	
			46	0	2	48	42		
			44	0	5	49	43		
7 a. m.	18°	16	23	1	2	26	24	24	24
			26	1	1	28	26		
			22	2	1	25	23		

occurring at 1 p. m. The primary minimum in the rate of cell-division occurs at 7 a. m. following the primary maximum, the secondary minimum at 3 p. m. following the secondary maximum.

Thus there are two regular waves of cell-division during each twenty-four hours, a more extensive wave extending from late in the afternoon until early in the morning, and occupying therefore practically all of the dark period; its crest occurs shortly before midnight. A less extensive but otherwise similar wave occupies the light period of the day extending from early morning until late in the afternoon. The crest of this wave occurs about noon. Both of these waves are remarkably regular with only the slightest secondary fluctuations.

The temperature of the air around the bulbs is recorded in the same diagram. It will be seen that there is no correspondence between these slight variations in temperature and the rate of cell-division.

(*b*) *Comparisons of other observations.* — FIGURE 2 includes curves derived similarly from two other series of root-tips. *Curve II* is introduced simply as another illustration of the normal type of curve such as *curve I*. Here the tips were cut less frequently so that all points of *curves I* and *II* do not correspond exactly.

The primary maximum occurs at 8 p. m., the secondary maximum at noon; the primary minimum at 5:30 a. m. and the secondary minimum at 5 : 30 p. m. It is unnecessary to give further examples of this character; in general it was found that under normal conditions of growth, cell-division was most active an hour or two before midnight and about noon, and was least active about 6 a. m. and 4 p. m.

Curve III in FIGURE 2 is introduced with its accompanying temperature curve to illustrate a very peculiar exception to this general rule. Cell-division here followed a perfectly normal

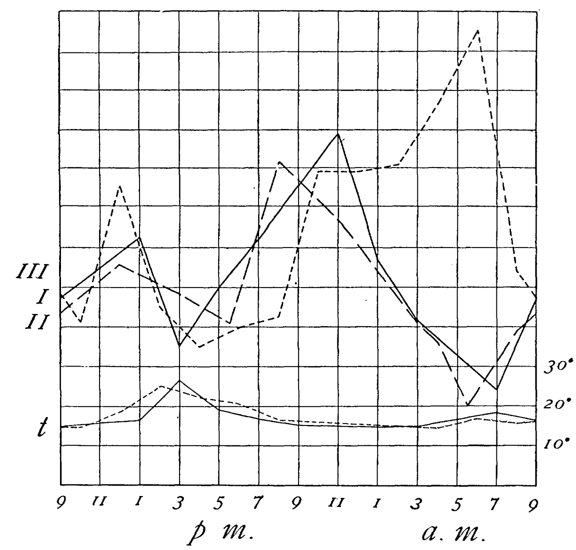

FIGURE 2. Cell-division in root of *Allium*. *t*, temperature.

course until just after the period of the primary maximum. Then instead of falling steadily to the primary minimum, it increased rapidly and reached a maximum far above that shown by any other series, at about the time when all other series showed the most pronounced minimum, *i. e.*, at 6 a. m. Then falling to its normal rate it proceeded after the typical fashion. That the rhythm of this plant was rendered abnormal in the prolongation of its most active period by some unknown conditions is evident. No other series showed any variation of this nature nor nearly of this extent. The curve is introduced here to show that when the usual rhythm of cell-division is interfered with, the normal rhythm

tends to recur when the disturbing factor is removed or overcome. The fact that the specific cause of the disturbance is unknown in this instance does not lessen materially the value of the evidence.

(*c*) *Comparison of observations on Podophyllum.* — We may now review briefly the results obtained from a similar examination of the rhythm of cell-division in the root of *Podophyllum.* This form was selected to be compared with *Allium* because it too had very large cells, rapidly and easily grown roots, and because it was a representative of a totally different subclass. The method used was the same as with *Allium,* except that the plants were

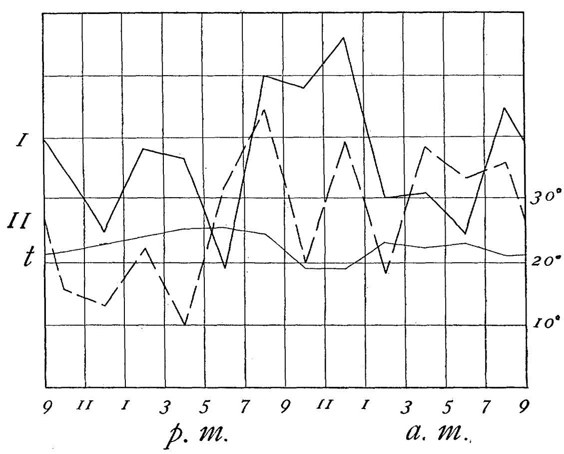

FIGURE 3. Cell-division in root of *Podophyllum.* *t,* temperature.

germinated in moist sphagnum, thoroughly rotted and washed.

The results obtained from two series of root-tips cut every two hours, are shown in FIGURE 3, together with the record of the temperature of the surrounding air. These curves show more extensive minor variations than did those of *Allium,* but it is remarkable how closely similar are the curves obtained from the two plants. Even the minor variations of the main curve are always of the same nature, differing only in extent, with the single exception of the period from 4 to 6 p. m. when one curve began to ascend shortly before the other.

Underlying these minor fluctuations there are the general waves of cell-division, most clearly marked in *curve I.* These show primary maximum and minimum at midnight and 6 p. m.

respectively, and less definitely-indicated secondary maximum and and minimum about 2 p. m. and 6 a. m. respectively. In *curve II* the secondary waves are obscured by the more extensive minor variations.

In general the character of these curves derived from *Podophyllum* is quite like those from *Allium*, maximal and minimal points occurring at approximately the same hours. The most valuable evidence offered by the *Podophyllum*, however, is that such rhythms are actually present in the rate of cell-division, and that the rhythms are definite enough to be susceptible of comparison.

We may now turn to the third line of evidence upon this subject.

B. EFFECTS OF SOLUTIONS

In order to test the effects of various solutions upon the rapidity and periodicity of cell-division, onions were germinated as before in moist sand or sawdust until the roots had reached a length of 50–75 mm. The bulbs were then placed over glass tumblers where the roots were immersed in tap-water and exposed to the daylight. Some were left in tap-water and examined at intervals, others were removed after being in water a few hours, to certain solutions, and examined at intervals.

The solutions used were: (1) 4.2 per cent. glucose in tap-water, as an example of a non-electrolytic solution; (2) 2 per cent. magnesium chloride, as an isotonic electrolytic solution; (3) 2.5 per cent. peptone.

(*a*) *Water*. — We may consider first the rate of cell-division of the tips placed in tap-water and subject to the illumination of the laboratory about 2.5 m. from north and west windows. An onion with roots about 50 mm. long, grown in sand, was placed over tap-water, in such a manner that the roots were completely immersed, at 10 a. m. and beginning at 2:30 p. m. tips were removed and examined. The numbers of dividing cells at irregular intervals during the succeeding thirty-two hours are represented in *curve I*, FIGURE 4. At 2:30 p. m., when the roots had been in water for four and one-half hours, the curve is much higher than is normal for that hour. During the afternoon it falls normally and from 8 p. m. until midnight when the curve usually rises to its greatest height, this curve shows only a very slight rise which

is rather an interruption in its rapid descent. After midnight there is continued descent until about noon when the curve ascends again to a maximum at 6 p. m. This maximum would have occurred normally at noon — just at the time when the ascent actually commenced. After the maximum at 6 p. m., which is the normal time for a minimal point, the curve falls again and was falling when observations ceased at 10:30 p. m.

The effect of removing the roots to water was at first a stimulation resulting in very rapid cell-division for a short time. This was followed by inhibition of cell-division and delay of its normal

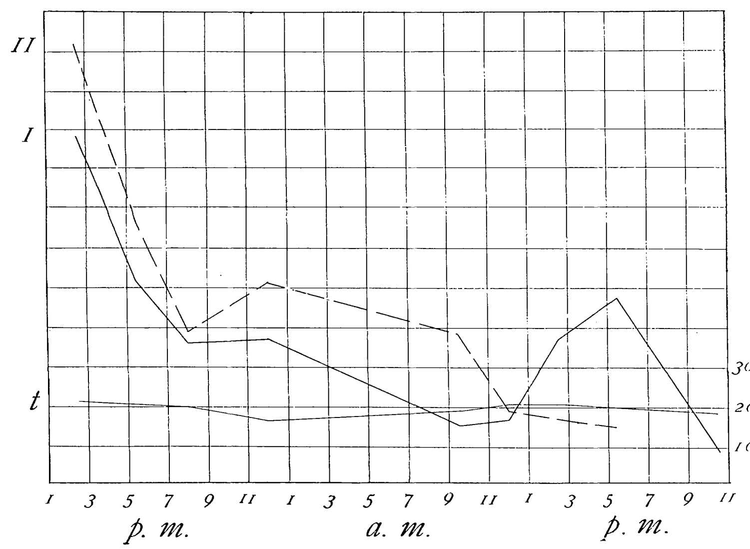

FIGURE 4. Elongation of root of *Allium* immersed in water (*I*), and in 4.2 per cent. glucose (*II*). *t*, temperature.

rhythms. The total number of dividing cells was very considerably reduced; the minimum which occurs normally about 6 a. m. was delayed until nearly noon, and the maximum which occurs normally at noon was delayed until 6 p. m. The inhibition of cell-division finally passed away partially and thirty-two hours after being placed in water (*i. e.*, at 6 p. m.) cell-division was again fairly rapid. Observations not included in the diagram were made at 10. a. m. on the two days following the last observation charted. These observations showed cell-division to be fairly active, the numbers of dividing cells being about 75–80 per cent. of the

normal. This is important as showing that very soon (about thirty-two hours in this instance) after being placed in a new or unusual environment the process of mitosis, at first seriously deranged, tends to recover its normal course. In other words the roots very soon become adapted physiologically to their new conditions of growth.

No correlation could be observed between variations in illumination or temperature, and cell-division. In this series the temperature varied only slightly (FIGURE 4).

(*b*) *Glucose.* — As before, the roots of an onion grown in sand until they were 50–75 mm. long were placed in tap-water at 9:30 a. m. At 2:30 p. m. they were removed to a 4.2 per cent. solution of glucose in tap-water and the tips examined at irregular intervals during the succeeding 27 hours. The result of the examination is shown as *curve II*, FIGURE 4. The first part of the curve is remarkably similar to *curve I*. In one respect it is more typical, namely in the rise just before midnight, where the tips in water showed only a pause in the descent. The latter part of the curve, however, is more atypical and does not give any sign of a delayed tendency to rise as was the case with the roots placed in water. This is probably to be correlated with the greater amount of stimulation resulting from the presence of an unusual substance in solution in such quantity. No later observation was made on this bulb.

The temperature was as in *curve I*, so that it is similarly true here that the variations in temperature and illumination of the laboratory seem to have no effect upon the frequency of mitosis.

(*c*) *Magnesium chloride.* — It was found that root-tips removed from water to a solution of $MgCl_2$ isotonic with the 4.2 per cent. solution of glucose, *i. e.*, a 2 per cent. solution $MgCl_2$, were seriously affected. The cells became vacuolated and somewhat shrunken and their condition became so abnormal in a few hours that there was no cell-division whatever in progress. Consequently roots were left in the $MgCl_2$ solution for only a short time and then were replaced in water.

For example, the roots of an onion grown in sand were placed in tap-water at 9:30 a. m. At 1:30 p. m. the tap-water was replaced by a 2 per cent. solution of $MgCl_2$ in tap-water and left

six hours; at 7:30 p. m. the roots were replaced in tap-water. Examination of the tips gave the results tabulated as *curve I*, FIGURE 5. At the time the roots were placed in the solution, cell-division was proceeding at about the usual rate for those tips similarly placed in water. After removal to the solution, however, cell-division decreased very rapidly. After replacing in water, cell-division recommenced and after a period of two hours we find the rate of mitosis gradually increasing until five hours after removal from the solution the rate was above the normal for roots placed in water, or about like those placed in glucose. Unfortunately the number of tips on this bulb was exhausted at this time and no further observation was possible.

(*d*) *Peptone.* — As a non-crystallizable, nutrient solution, a 2 5. per cent. solution of peptone in tap-water was used. As before,

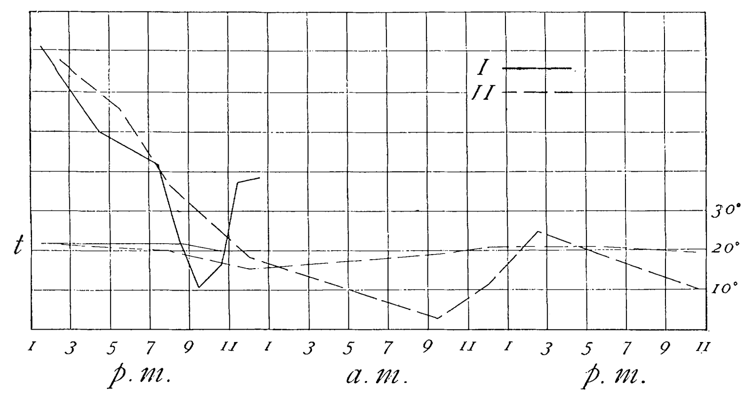

FIGURE 5. Elongation of root of *Allium* immersed in 2 per cent. $MgCl_2$ (*I*), and in 2.5 per cent. peptone (*II*). *t*, temperature.

roots were removed from sand to water at 10 a. m. At 2:30 p. m. they were transferred to the peptone solution and examined at intervals during a period of thirty hours. The rate of mitosis in these tips is shown graphically in *curve II*, FIGURE 5. In general it is very similar to the curves of tips in water and glucose, especially during its later course. In its earlier portion it lacks the pause or rise just before midnight. Cell-division almost stopped after 16–20 hours in the solution.

As already stated the general result of placing growing roots in liquids or solutions is to decrease the rate of cell-division and to

delay its normal rhythm. Upon comparing the effects of various types of solutions certain tentative inferences may be drawn. The effect of a non-electrolytic solution (glucose) is not different from that of tap-water alone; in this instance the plant did not tend to recover its normal rhythm, *i. e.*, to adapt itself, as did the plant placed in water alone, although the first effect was not quite as marked.

The effect of a brief immersion in an electrolytic solution (magnesium chloride), however, was the almost complete stoppage of cell-division; recovery commenced shortly after removal to water. The effect of a solution of peptone is to cause a reduction of the rate of cell-division greater than that produced by water alone; the rhythm is not affected dissimilarly.

From the small number of observations made it is obviously impossible to make a definite statement regarding the specific effect of any of these solutions upon cell-division. The purpose of placing the roots in these solutions was not at all to test their specific effects, but to determine the effects of liquids in general upon the rate and rhythm of cell-division; rather to see whether the normal rhythm was actually a definite thing and whether it would be maintained or modified under abnormal external conditions. From this point of view the evidence from the experiments was decisive and positive. That the rhythm is a definite occurrence is sufficiently indicated by the uniform periods of increase and decrease in the rate of mitosis in all series of tips whether under normal or abnormal conditions. The result of placing the tips in liquids is to prolong the rhythm and so to delay the maximal and minimal points, although these tend to recur at the normal hour even under abnormal conditions, and to decrease the rate of cell-division. This accords with the observations of Sachs,* Wacker,† and others that the roots of land plants are shorter when grown in water — undergoing a sort of retardation of growth.

It is noteworthy that in none of the series could there be seen any direct effect of change in temperature. The variations however were slight, and it almost goes without saying that wide varia-

* Sachs, J. Arbeiten Bot. Inst. Würzburg, 1 : 385.

† Wacker, J. Die Beeinflussung des Wachsthums der Wurzeln durch das umgebende Medium. Jahrb. Wiss. Bot. 32 : 71–116. 1898.

tions in temperature would have a very marked effect upon the rate of mitosis. Nor could there be observed any effect upon mitosis of changes in the feeble illumination of the laboratory. Normally, of course, the root is in a constant condition of darkness and it is therefore worthy of note that the rhythm in the varying illumination of the room had no definite effect upon the rate of cell-division.

Finally then, summing up in regard to periodicity of cell-division, we may say that there is a definite and regular rhythm in the root of *Allium*. Under normal conditions of growth, cell-division is most active just before midnight and just after noon and is least active about 6 a. m. and 4 p. m. There may be variations from these times within limits of about two hours.

Very little is possible in the way of comparing these results with the work of others. Famintzin,* Strasburger † and others have noted that in most algae cell-division is most rapid at night. Lewis ‡ in a very brief communication noted that cell-division in the root of the onion growing in water and exposed to the light, was most rapid about noon and slowest about 4 p. m., while in roots growing in water but in a vessel painted with lamp-black cell-division was slowest at midnight and fastest about 4 p. m. Insufficient data are given concerning the conditions under which these results were obtained to enable us to judge of the value of these observations.

5. Daily periodicity of elongation

The observations upon the elongation of the root of *Allium* were all made upon the roots of bulbs of uniform size and with approximately equal numbers of roots, *i. e.* about six. The length of the roots varied between 35 and 50 mm. at the beginning of the observation. The roots had been grown entirely in the dark, the bulbs not having been exposed to the light after germination was begun, so that no rhythmic character of elongation could be due to the direct influence of alternating illumination. The tempera-

* Famintzin, A. Die Wirkung des Lichtes auf Algen und einige andere ihnen nahe verwandte Organismen. Jahrb. Wiss. Bot. **6**: 40. 1867.

† Strasburger, E. Zellbildung und Zelltheilung. 3 Aufl. 171. 1880.

‡ Lewis, A. C. Contributions to the knowledge of the physiology of karyokinesis. Bot. Gaz. **32**: 424–426. 1901.

ture in the dark room varied within narrow limits only and the temperature within the pot containing the roots must have been nearly constant. Thus the factors of varying illumination and temperature were ruled out.

The facts that the roots were of different lengths and that they showed different total amounts of elongation during the periods of observation show that the roots were not all in the same phase of the grand period of growth. This has to be taken into account constantly in comparing the different curves of growth, for some will show a general upward course, others a downward or even course, depending upon the relative portion of the grand period of

TABLE II

ELONGATION OF THE ROOT OF ALLIUM. TOTAL ELONGATION, 24 HRS. = 16.1 MM.

Time of observation.	Elongation in one hour, in micrometer spaces.	Temperature.	Time of observation.	Elongation in one hour, in micrometer spaces.	Temperature.
9 a. m.	10.00	21.0°	9 p. m.	8.00	20.5°
10 "	8.75	20.5°	10 "	8.00	"
11 "	8.50	"	11 "	6.00	"
12 m.	10.00	"	12 n.	7.00	"
1 p. m.	10.00	"	1 a. m.	8.00	"
2 "	9.00	"	2 "	8.00	20.0°
3 "	9.00	"	3 "	7.00	"
4 "	9.50	"	4 "	7.00	"
5 "	8.75	"	5 "	8.00	"
6 "	8.75	"	6 "	9.00	"
7 "	8.75	"	7 "	8.00	"
8 "	9.00	"	8 "	9.00	"

growth which is represented. Thus while in the daily curves of elongation the actual maximal and minimal points coincide, their relative heights might vary somewhat.

(*a*) *Typical observation.* — We may take as an example a record of the elongation of the root showing the hourly elongation in micrometer spaces (Leitz horizontal microscope) as given in TABLE II. This is expressed as a curve in FIGURE 6, *curve I*, where the observed elongation is multiplied by four to bring out better the form of the curve.

There are seen to be apparently three maxima and three minima in the rate of elongation. The most extensive maximum (primary maximum) has its apex at 4 p. m. The primary minimum has its apex at 10 p. m. Secondary maxima occur at 3 a. m.

and 7 a. m. and secondary minima at 5 a. m. and 1 p. m. These secondary maxima and minima are relatively much less marked than the primary waves and, occurring as they do so near together, it is likely that they are to be considered properly as minor fluctuations on a broader maximal wave reaching its height (secondary maximum) about 7 a. m.; the secondary minimum would then be found about noon. The other irregularities in the curve are quite slight.

The observations on some of the roots were made only bi-hourly, therefore for purposes of comparison later, we may write this curve as if observations had been made bi-hourly, as *curve I*, FIGURE 7. The result, of course, is to reduce the irregularities of the curve and slightly to alter the times of the apices of the princi-

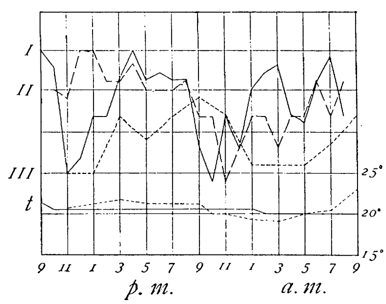

FIGURE 6. Elongation of root of *Allium*. *t*, temperature.

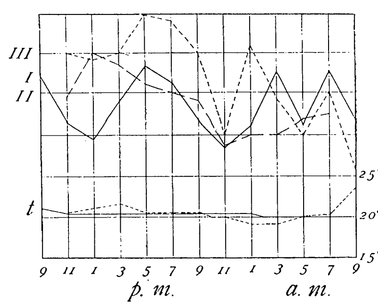

FIGURE 7. Elongation of root of *Allium*. *t*, temperature.

pal waves, *e. g.* the primary maximum now appears at 5 p. m. instead of 4 p. m., the primary minimum at 11 p. m. instead of 10 p. m.

(*b*) *Comparison of other observations.* — *Curve III*, FIGURE 7, illustrates the rate of elongation of a root observed bi-hourly. The general appearance of this curve is somewhat modified from the fact that it was taken after the maximum of the grand period of growth had been passed and consequently the rate of elongation is lower at the end than at the beginning of the observation. The actual hours of maxima and minima are, however, almost exactly as in the curve just described. That is, there are primary maximal and minimal points at 5 p. m. and 11 p. m. respectively, and secondary maximal and minimal points at 7 a. m. and 1 p. m. The temperature varied more during this observation than during

the preceding one, but still no correlation is observable between variations in temperature and rate of elongation.

Another record, *curve II*, FIGURES 6 and 7, was made at the same time as *curve I* and under identical conditions. The points of dissimilarity between the two are of degree rather than of kind. There is just the same pronounced minimum at 11 p. m. The primary maximum however occurs somewhat earlier, that is at 1 p. m. The secondary maximum occurs at the usual hour. The secondary minimum about noon is not very clearly marked but is indicated at 11 a. m. On the whole the secondary waves are all less definite than in the curves previously described.

Finally, another record made under conditions identical with those of *curve III*, FIGURE 7, must be considered. This record is illustrated as *curve III*, FIGURE 6. This is the only root observed during the ascent of the grand period of growth and most of its very evident peculiarities may be explained in connection with this fact. The rate of elongation reaches its primary maximum at 9 p. m. Another maximum is marked definitely at 3 p. m. and the rate was increasing again steadily when the observation ended at 9 a. m., as if to mark another maximum. A very extensive minimum occurs between 1 and 5 a. m., and at the beginning of the observation a minimal period extends from 9 a. m. to 1 p. m. The features of the curve are all remarkably regular and well marked. The total amount of elongation during the 24 hours was only 13.5 mm. There is no correlation between the rate of elongation and the comparatively slight variations in temperature.

The principal peculiarities of this record are due to the appearance of the primary maximum and minimum about five hours later than normally and to the absence of the secondary waves in the early morning. A possible explanation of these peculiarities may be the following. From the comparative levels of the beginning and end of the curve and from the small amount of elongation of the root, we are evidently concerned here with a root which has not yet reached the maximum of its grand period of growth. Such a young root is growing with a greater vigor, has a greater momentum so to speak, and as a consequence is not affected by stimuli (external or internal) which would affect roots

growing with less potential, such for instance as are on the descent from the maximum of the grand period. Ordinary stimuli would produce either a delayed reaction or none at all. Therefore the daily maximal and minimal periods are delayed and the stimuli usually producing the secondary waves are inoperative.

Summarizing then in regard to elongation we may say that there is a daily rhythm of elongation of the root of *Allium* which occurs in the absence of variation in illumination and independently of slight variations in temperature: this rhythm must be the result of internal factors. The daily rhythm is such that elongation is most rapid about 4 or 5 p.m. and slowest about 11 p. m. Besides these primary waves there are secondary points, the secondary maximum occurring about 7 a. m. and the secondary minimum about noon.

It will be well to compare, at this point, these results on *Allium* with those derived from the study of other subterranean organs. As mentioned in the introduction to this paper,* the only records are those of Strehl and MacMillan. Strehl found that there was a single maximal and minimal wave in the rate of elongation every twenty-four hours, the rate being highest about midnight and lowest about noon. His curves, which represent observations extending over several days, show admirably the varying appearance of the daily curve depending on the phase of the grand period which it represents. Strehl's observations were made upon the roots of *Lupinus*, and the conditions under which they were growing were far from normal; *i. e.*, the roots were growing in water and were contained in glass placed only 1.5 m. from a west window where they would be subject to moderately strong illumination.

In MacMillan's observations upon the growth-periodicity of the potato-tuber, the plants were under normal conditions of growth and the results are entitled to more weight. Unfortunately, as he himself states, his observations were of a fragmentary nature and were to be considered rather as a preliminary report. MacMillan concluded that the rate of increase in diameter of the potato-tuber occurred rhythmically, with one, two, or perhaps more maxima in twenty-four hours. He believes that the tuber

* See page 530.

has an independent rhythm of its own in addition to a rhythm induced by that of the stem and other aërial parts, and offers some tentative explanations of both of these. The fact that here interests us particularly is that there is frequently a double maximum in the rate of growth during the twenty-four hour period. Thus far there is agreement with the root of *Allium*; how much farther this agreement extends can not be stated, for MacMillan does not mention any hours at which these maxima occur.

We may also compare briefly at this point, the rhythm of elongation of the aërial parts. Observations upon *Allium* have been made by Stebler * on the elongation of the leaf. Stebler found that the maximum of growth coincides with the period of greatest light intensity (12–4 p. m.), growth being slower during the night, the minimum occurring between 12 and 4 a. m. His curves show this single wave very definitely. Many others working on other plants have found similarly a single daily wave of growth, although the actual periods of maximum and minimum do not always correspond by any means. Thus, for example, Sachs † found the elongation in *Dahlia* to be most rapid in the early morning (6–9 a. m.) and the slowest in the evening, (3–6 p. m).; Prantl ‡ in *Cucurbita* found the maximum elongation to occur about 3 p. m. (12–6 p. m.) and the minimum from 3 to 6 a. m.; and, to complete the possibilities, MacDougal § found that in the leaf of *Hyacinthus* the maximum of elongation occurs between midnight and 4 a. m. and the minimum about noon. The only uniformity here is the single maximum and minimum each day.

Godlewski || at first observed only a single wave of elongation in the epicotyl of *Phaseolus*, but later he found that there were definitely two maximal and two minimal periods during twenty-four hours. The minima occurred morning and evening

* Stebler, F. G. Untersuchungen über das Blattwachsthum. Jahrb. Wiss. Bot. **11**: 47–123. 1878.

† Sachs, J. Arbeiten Bot. Inst. Würzburg. **1**: —.

‡ Prantl, K. Arbeiten Bot. Inst. Würzburg, **2**: —.

§ MacDougal, D. T. Text-book of plant physiology, 295. New York. 1901.

|| Godlewski, E. Studien über das Wachsthum der Pflanzen. Abh. Krakauer Akad. Wiss. Math-Naturw. Classe, **23**: 1–157. (Abstract by Rothert, Bot. Centralblatt, **55**: 34-40. 1893.)

and the maxima day and night; the relative intensities of the maxima and minima were inconstant even in the same plant. This is apparently the only recorded instance of a double daily wave of growth in an aërial part, of such a nature as is found in the root.

6. Relation between cell-division and elongation

The actual relation in time between cell-division and elongation can be seen most easily by reference to FIGURE 8, where there are composite curves of all my records of normal cell-division and elongation reduced to similar proportions. It is at once evident that the rhythms agree in time but always occur in opposite direc-

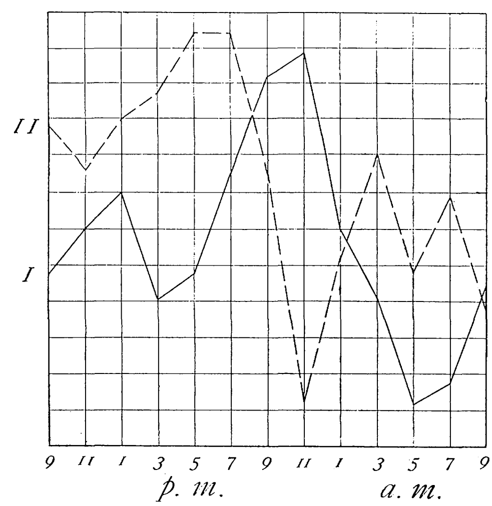

FIGURE 8. Composite curves of cell-division (*I*) and elongation (*II*).

tions, *i. e.* when elongation is at a maximum cell-division is at a minimum and vice versa. The primary maximum of cell-division corresponds perfectly with the primary minimum of elongation at 11 p. m. The primary maximum of elongation corresponds with the secondary minimum of cell-division about 5 p. m., while the primary minimum of cell-division occurs at the time of the secondary maximum of elongation at 5 a. m. In general, throughout, the curves have opposite directions. The only real exception occurs between 3 and 7 a. m. when, as already noted, the decreasing rate of elongation can hardly be considered a constant

feature; it is quite likely that the rate of elongation increases uniformly from 11 p. m. until 7 a. m.

These time relations, however, do not lead to the understanding of any causal relation more definite than that expressed by Ward,* that the energy of the cell is not sufficient to enable it to continue its growth and at the same time form new cellular elements; therefore when the cell is dividing and the new cell-wall forming, etc., the energy of the cell is diverted from its other growth-processes and elongation is arrested. Rhythmic activity resulting from the uniform action of stimuli or forces is quite universal and it may be that here in the root where external conditions are practically uniform, we are dealing with a rhythm which is not related directly to the external environment, but which results from the activity of the root itself, *i. e.*, is internal in its origin. There can be no doubt that the rhythm in the stem is to a greater or less extent an induced one, and it is difficult to believe that the pronounced rhythm of the stem is without influence upon the root; yet in the roots of these bulbs without growing aerial organs, definite rhythms are established. We should know whether this rhythm of the root is maintained unchanged after the development of the leaves with their marked daily rhythm. With our present limited information we are hardly justified in making assumptions as to what are the causal relations between cell-division and elongation, both to each other and to the other vital activities of the plant.

* Ward, H. M., *loc. cit.*

COLUMBIA UNIVERSITY,
June 1, 1904.